

Symposium on Microbial Insecticides

III. Fungal Parasites of Insects and Nematodes¹

DAVID PRAMER

Department of Agricultural Microbiology, Rutgers, The State University, New Brunswick, New Jersey

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INTRODUCTION

Fungal parasites of insects and nematodes are too important to be treated superficially, and current knowledge of the subject is much too extensive for complete coverage here. I have no alternative, therefore, but to deal in detail with selected aspects of my topic, and to hope that in so doing, I will focus attention on a field of fascination and future promise in microbiology. Here, then, are gleanings from a voluminous literature that pertains to fungal parasites of insects and nematodes. No claim is made for originality of either content or exposition. My own investigations are concerned with a group of microorganisms known as the nematode-trapping fungi. These will be considered subsequently, but it must be understood that the major portion of my text describes the work of others on organisms with which I have had little or no personal experience.

INFECTIONS OF INSECTS

Fungal parasites are unique in the history of insect pathology (34). The very first experimental proof that a disease of animals can be caused by a microorganism was the demonstration, in 1835, by Agostino Bassi that muscardine of the silkworm was a fungal infection. Moreover, it was a fungus parasite of the cockchafer that Metchnikoff used, in 1879, to first demonstrate that a

microbial disease of an insect of economic significance can be established intentionally. From these beginnings, insect pathology has developed as an experimental and applied science. Today, many representatives of the fungi are known to cause disease in insects. There are numerous parasites of insects among the Phycomycetes, Ascomycetes, and Deuteromycetes, but the number of Basidiomycetes known to infect insects is relatively small. Varying degrees of parasitism are recognized, and many fungus-insect relationships have been described. It is not practical to attempt here a discussion of all of the species of fungi involved or the infections they cause, but some essential relationships will be exemplified by the following consideration of a few of the more completely described insect mycoses.

Entomophthora

The Entomophthorales constitute an order of Phycomycetes that is divided into six genera, one of which, *Entomophthora*, contains more than 100 species known to parasitize insects. Entomophthorales infections have been reviewed by Steinhilber (33) and, more recently, by MacLeod (23). Insects representative of seven different orders (Orthoptera, Homoptera, Hemiptera, Coleoptera, Lepidoptera, Diptera, and Hymenoptera) are attacked by *Entomophthora* species, and susceptibility to infection is shared by larvae, pupae, and adult.

Infection results when an airborne spore germinates and penetrates the exoskeleton of an insect. Vegetative growth occurs in the living host, but hyphal extension is limited, and there is fragmentation of filaments into their component cells. These develop into hyphal bodies which are short, multinucleate, and variable in form and size.

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Hyphal bodies increase in number by fission and budding, and, ultimately, fill the insect hemocoel. As the infection progresses, insects become sluggish, and eventually lose their power of locomotion. They may drop to the ground or come to rest on the underside of branches or leaves. Frequently, hyphae penetrate the body surface and form rhizoids which anchor the cadaver firmly to the site at which death occurred. Shortly after death of the insect, the hyphal bodies give rise to spores. These may be of the resting (chlamydospores), sexual (zygospores), or asexual (conidia) type, but, under optimal conditions of temperature and moisture, it is conidia that are formed predominantly. Conidiophores develop rapidly, penetrating the less resistant portions of the exoskeleton, and conidia may be recognized externally on the body surface, within a few hours of death of the insect. *Entomophthora* conidia and conidiophores appear to absorb water differentially, which causes pressure to be exerted and the spores to be discharged violently into the air. By this mechanism the parasite is dispersed over considerable distances, and the probability of renewed contact with a living host is increased.

MacLeod (23) emphasized that great difficulty is encountered by investigators who attempt to infect healthy insects with *Entomophthora*. It appears that conditions which induce natural infections and a highly contagious state are unknown and, therefore, cannot be reproduced at will in the laboratory. *Entomophthora* organisms are not obligate parasites. They develop saprophytically on a variety of complex organic substrates (30). Some species have no requirement for an exogenous supply of vitamins or other accessory growth factors, and can be cultivated on simple media that are chemically defined (31, 38). However, much remains to be revealed regarding the nutrition of *Entomophthora*. Also, information (15, 16, 30) concerning the influence of environmental factors on growth, morphogenesis, and pathogenicity is limited, and must be extended greatly if these fungi are to be understood, and, through understanding, be exploited for the biological control of insect pests.

Cordyceps

Ascomycetes are divided into two subclasses, the Hemiascomycetes and the Euascomycetes, in which ascocarps are present and absent, respectively. The genus *Cordyceps*, which is included in the order Hypocreales, subclass Euascomycetes, contains approximately 200 species, the majority of which parasitize insects. Members of the genus have been isolated from seven different orders of insects (Diptera, Hymenoptera, Coleoptera, Lepidoptera, Hemiptera, Isoptera, and Orthoptera)

and from spiders, and they are well characterized taxonomically. Insects parasitized by *Cordyceps* species are bizarre in appearance and attracted the attention of early naturalists. Steinhaus (33) cites Torrubia, a Franciscan friar, who, in 1749, on finding some dead wasps in a field near Havana, wrote, "from the belly of every wasp a plant germinated, which grows about five spans high." It is of interest to note also that some *Cordyceps*-infected insects are used as food and for medicinal purposes in China (17). Our present knowledge of the taxonomy, morphology, physiology, and pathogenicity of *Cordyceps* was most recently reviewed by McEwen (26).

It is characteristic of *Cordyceps* that the sclerotium located within the infected insect gives rise to a stroma or aerial structure, consisting of an outer layer of short lateral hyphal branches arising from a central core of parallel and intertwined filaments. The sterile, erect portion of the stroma usually terminates in an enlarged fertile head that contains perithecia and may be brightly colored. Stromata vary in length from 1.5 to 300 mm, and this exposed portion of *Cordyceps* was described in early writings as a plant or tree developing from a "bug's belly."

If spores of *Cordyceps* germinate on the surface of a suitable host, the integument of the insect is penetrated, and, through a process of fragmentation, hyphal bodies are produced in the hemocoel. The hyphal bodies multiply by budding. They increase in number in the blood and eventually fill the entire body cavity. Death of the host occurs, but there is continued growth of the fungus, and the compacted hyphal strands form the sclerotium from which the stroma arises (25, 33). Most species of *Cordyceps* appear to be host specific, but exceptions are known. *C. militaris* is notable in this regard, and has been reported as parasitic on 13 genera of Lepidoptera, on coleopterous pupae, and on some Hymenoptera (37). There have been a limited number of attempts to culture these fungi on laboratory media. Huber (18) reported that *C. militaris* grew well on a medium containing glycerol as the sole source of carbon and energy. The fungus required no accessory growth factors and was capable of utilizing both inorganic and organic nitrogen. At present, there is great need for information relating nutritional and environmental conditions to infectivity, growth, and sporulation of *Cordyceps* species.

Septobasidium

Of the few Basidiomycetes that parasitize insects, members of the genus *Septobasidium* are the most noteworthy. There are approximately 160 known species that live in balanced associa-

tion with about 20 species of scale insects. Couch (3) studied in detail this fungus-insect relationship, and it is from his treatise on the genus *Septobasidium* that most present information is derived.

The *Septobasidium* colony develops best on the undersurface of the lower branches of trees, and is composed of a series of irregular concentric rings of annual growth. It can be as large as 30 cm in diameter and 1.0 cm thick. Within the colony are numerous tunnels and chambers which contain the scale insects. The insects feed on the woody host plant, and the fungus provides them with shelter. However, some of the insects are parasitized and nourish the fungus. The young scale insect contacts the yeastlike bud of the germinating basidiospore, which adheres to the body surface and initiates infection. Hyphae of the fungus enter the circulatory system of the living insect and there form numerous coiled haustoria. Growth of the fungus within the parasitized insect is exceedingly slow. Infected insects continue to feed on the host plant, but they are dwarfed and incapable of reproduction. Many of the young insects escape infection and remain to reproduce the species, while those that are parasitized nourish the fungus which provides shelter for all. Unless insects are infected when young, they remain free from the fungus. Thus, fungus and insect live together interdependently.

Beauveria

Beauveria species are the best known of the Deuteromycetes that parasitize insects. They are widespread and frequently the most abundant of fungi isolated from collections of dead insects. *B. bassiana* is the agent responsible for muscardine of the silkworm, the first disease of animals demonstrated to be of microbial origin, and referred to previously. Although *B. bassiana* proved costly to the silk industry, it can parasitize more than 30 different insect species, including the corn borer, the codling moth, and the potato beetle, and is a favored subject for study by those concerned with biological control (10, 35, 36). The morphology and taxonomy of *Beauveria* species were reviewed thoroughly by MacLeod (21), and *Beauveria* diseases of insects were described and discussed by Steinhaus (33) and Madelin (24).

The infective unit in *Beauveria* diseases is the spore. Tracheal openings or the digestive tract are possible routes of infection, but it appears that in most instances spores contaminating the surface of the insect germinate and penetrate the integument. Within the body cavity of the host, the fungus continues to develop. Hyphal bodies are formed, blood cells are destroyed, and circulation is decreased in rate. Glandular structures and

ganglia may be invaded, and the fungus frequently enters tracheae, impairing respiration. An infected insect moves sluggishly and fails to respond to external stimuli. Eventually, there is general paralysis, followed by death and mummification of the host. Colonization of the dead insect culminates in the formation of a mass of mycelium within the brittle integument. In a dry atmosphere, the fungus may lie dormant for prolonged periods, but soon after exposure to moist air a mycelium develops over the surface of the insect, and conidia are produced abundantly within 1 or 2 days. Since the conditions that favor sporulation are similar to those that favor infection, the disease is transmitted from insect to insect and perpetuated within a population.

Beauveria species grow readily on a medium that contains glucose, ammonium-nitrogen, and inorganic salts only (22), and there is no diminution of virulence with continued transfer of pure cultures in the laboratory (19). Spore germination and infection of host insects is favored by high humidity and is influenced by temperature (24). Penetration of the insect integument is usually attributed to enzymatic mechanisms. Huber (18) demonstrated that *B. bassiana* hydrolyzed chitin, but in the presence of more simple substrates chitinase production by the fungus was suppressed. It is possible that infectivity is a function of the nutrient status of the environment.

INFECTIONS OF NEMATODES

I wish now to direct your attention from fungi that parasitize insects to those that parasitize nematodes. Nematode parasites comprise a heterogeneous group that includes representatives from each of the major classes of fungi. They can be divided arbitrarily into the nematode-trapping fungi and the endozoic fungal parasites (9).

Nematode-Trapping Fungi

Most of the nematode-trapping fungi are classified within the order Moniliales of the Deuteromycetes, but some are Phycomycetes and at least one is a Basidiomycete (6, 7). Although they are taxonomically diverse, the nematode-trapping fungi are ecologically a natural group, united by their adaptation to the predaceous habit (4, 5). They are able to capture, kill, and consume worms of microscopic dimension, and, for this purpose, produce specialized hyphal structures that are truly remarkable. These organelles of capture vary greatly in form, but they trap nematodes by either adhesion or occlusion.

In some species, the mycelium gives rise to one-celled processes, short branches, or spherical knobs on aerial hyphae. In other species, branches elongate and curl, and there may be considerable

anastomosis to form a three-dimensional system of hyphal loops. In every case, these structures are coated with a sticky secretion of unknown composition, and they capture nematodes by adhesion and entanglement. There are two types of traps that function mechanically: the nonconstricting and the constricting ring. Both are composed of three cells which join to form a closed ring at the end of a short mycelial branch. A nematode entering the nonconstricting ring, and attempting to force its way through, becomes firmly wedged and is unable to escape. Constricting rings are active rather than passive. When a nematode enters, the cells that comprise the ring swell to approximately three times their normal volume, obliterating the opening and constricting the nematode so that it cannot escape. Ring closure is a thigmotactic response and does not require more than 0.1 sec. Whatever the species of fungus or the trapping mechanism involved, a captured nematode struggles for a time, dies, and is then penetrated by fungus hyphae which ramify throughout the carcass, digesting and absorbing its content. Under favorable conditions, nematodes may be captured in large numbers, but the actual cause of death of a trapped worm is uncertain. It may be due to exhaustion or physical damage, but the production by the fungus of a toxin is a possibility that cannot be ignored (27, 32).

Nematode-trapping fungi are not obligate parasites. They grow readily in pure culture on a variety of laboratory media, but some species do not produce traps in the absence of prey. I have had recent opportunity to summarize the results of work in my laboratory concerned with the nutrition and biochemical basis of morphogenesis in nematode-trapping fungi (29). Repetition and extension here are tempting but not justified. There have been many laboratory, greenhouse, and field trials performed in an effort to exploit the activity of predaceous fungi for the control of nematode pests (8, 9). The results are promising, but have never been decisive. Empirical attempts at biological control will continue to be made, but the work will be easier and the probability of success increased greatly if future tests have a rational basis derived from fundamental investigation and experimental fact (28). In testimony to the validity of this statement, I will limit my discussion of the many fungi that do not form traps, but are endozoic parasites of nematodes (9), to species of the genus *Harposporium*. The tale I tell is meant to emphasize the necessity of fundamental fact as armament for the strategist who wishes to employ biological agents in a meaningful attack on insect and nematode pests.

Harposporium

Nematodes infected with *Harposporium* were first encountered by Lohde in 1874 (20), but they occur commonly in a variety of habitats, including soil, moss cushions, and decomposing plant and animal residues. Conidiophores of the fungus develop from a vegetative mycelium within the host. They penetrate the integument, and, in air, bear crescentric or sickle-shaped spores with pointed tips. Several opinions have been expressed regarding the mechanism of infection by *Harposporium*. The spores appeared too large to enter host species by the oral route, and this possibility was rejected. Morphology was related to mode of infection, and it was suggested by some that the pointed tip, on impact, served to impale the nematode on the spore. However, most investigators agreed that a drop of mucilage secreted at the spore tip acted, on contact, to glue the spore to the outer integument of the nematode. In 1959, with this dogma in mind, Feder and Duddington (11) decided to test the ability of *Harposporium* to control a nematode disease of plants. Their decision was undoubtedly based on the observation that *Harposporium* species were widespread and apparently successful obligate parasites. Moreover, there was need to compare endozoic fungus species with nematode-trapping species as agents of biological control.

An obligate parasite will develop in only living-host tissue. Therefore, to obtain adequate supplies of *Harposporium* for laboratory and field studies, Feder and Duddington (11) reared, infected, and then lyophilized thousands of nematodes. Regrettably, their efforts were in vain, for, as they labored to accumulate nematode tissue infected with *Harposporium*, Aschner and Kohn (1) reported that the organism is not an obligate parasite, that it has no specialized nutrient requirements, and that it will develop readily on a glucose-inorganic salts medium containing ammonium-nitrogen. *Harposporium* parasitizes nematodes of the *Rhabditis* group that feed on particulate matter, but is unable to infect stylet-bearing species of the genus *Dorylaimus* that consume fluids only. To determine the mode of invasion of host by the fungus, Aschner and Kohn (1) examined early stages of infection of rhabditic nematodes. Spores did not adhere to living worms, and the fungus was never observed to penetrate the nematode integument. Infection of nematodes by *Harposporium* species proceeds by the oral route only. *Harposporium* spores are long but they are slender and can be swallowed. Entrance is facilitated by the nematode itself, for the worm modifies the dimensions of its alimentary tract when ingesting particles that vary in size and shape.

These observations are of the utmost significance and necessitate a complete re-evaluation of *Harposporium* species as agents for the biological control of nematode pests. The fact that these fungi are widespread may be a reflection of their saprophytic rather than their parasitic ability. Moreover, most economically important species of nematodes have mouth parts that are modified to form a needlelike stylet for sucking. Since the stylet opening is less than $1\ \mu$ in diameter and will not pass spores, no successful control of a population of plant parasitic nematodes can be expected from *Harposporium*, or from any other fungal parasite that must enter its host by the oral route.

COMMENTS CONCERNING MICROBIAL CONTROL

There is at present a great desire to direct microbial parasites against economically important agricultural pests. This interest derives in part from the fact that field collections of insects and nematodes frequently contain infected specimens. It is due also to recurring reports in which microorganisms are described as responsible, in nature, for dramatic reductions in the density of insect populations. However, present concern must be attributed primarily to the undesirable effects of persisting residues of chemical pesticides and to recognition of an increase in both degree and incidence of resistance of pests to these chemicals. The numerous individual attempts to control insects and nematodes with fungal parasites cannot be considered here. Fortunately, they have been compiled and recently reviewed by others (2, 8, 9, 12, 13, 14, 33, 35, 36). The usual approach to microbial control has been empirical. Frequently, published reports reflect a lack of thoroughness in experimental design, procedures are not described fully, and results are often indeterminate or inconclusive. Nevertheless, Baird (2), after reviewing the enormous quantity of work that has been done in this field, cites as successful some 41 attempts to employ fungi for the control of 28 different insect pests, and the potentialities of many of these fungi are in the process of being reappraised (13, 14).

Microbial control has intellectual and popular appeal and support, but it is among the most difficult of tasks to accomplish. The degree of success achieved is frequently a direct function of available knowledge of the system under study. Steinhäus (33) has emphasized that disease is to be regarded as a process and not a thing. It is necessary that the factors which initiate and control this process be identified, and that investigations be performed to reveal the biochemical basis of the host-parasite relationship. This is not meant to imply that understanding will assure success,

but the cause of an applied biologist interested in using microorganisms for the control of pests will be advanced greatly if he bases his efforts on reliable information concerning both parasite and host, acting individually and as an integrated unit under a variety of environmental conditions. Much remains to be accomplished, but I wish to stress that there is more to fungal parasites of insects and nematodes than their application. They comprise an engaging group and provide the interested investigator with a unique opportunity to apply the principles and techniques of contemporary microbiology and to contribute to our understanding of fungi and fungal infections.

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